# Serum Concentrations of Estrogens, Sex Hormone Binding Globulin, and Androgens and Risk of Breast Hyperplasia in Postmenopausal Women

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## Abstract

Objective: **We sought to determine whether serum concentrations of estrogens, androgens, and sex hormone binding globulin in postmenopausal women were related to the presence of mammary hyperplasia, an established breast cancer risk factor.**

Methods: **Study participants provided serum before breast biopsy or mastectomy in three hospitals in Grand Rapids, Michigan, between 1977 and 1987. A total of 179 subjects with breast hyperplasia were compared with 152 subjects with nonproliferative breast changes that are not associated with increased breast cancer risk.**

Results: **The odds ratios (OR) associated with the three upper quartiles of estradiol in comparison with the lowest quartile were 2.2 [95% confidence interval (95% CI) 1.1-4.6], 2.5 (95% CI, 1.1-5.3), and 4.1 (95% CI, 2.0-8.5; $P_{trend}$ = 0.007). The corresponding ORs for bioavailable estradiol, estrone, and estrone sulfate were of generally similar magnitude ($P_{trend}$ = 0.003 for bioavailable estradiol, 0.0004 for estrone, and 0.0009 for estrone sulfate). Relative to women concurrently in the lowest tertile for serum estradiol, estrone, and estrone sulfate, women concurrently in the highest tertile for all three hormones had an OR of 5.8 (95% CI, 2.2-15.2). Serum concentrations of sex hormone binding globulin, testosterone, dehydroepiandrosterone, androstenedione, and androstenediol were not associated with risk of hyperplasia.**

Conclusions: **Serum concentrations of estrogens, but not of androgens or sex hormone binding globulin, were strongly and significantly associated with risk of breast hyperplasia in postmenopausal women, suggesting that estrogens are important early in the pathologic process towards breast cancer.** (Cancer Epidemiol Biomarkers Prev 2005;14(7):1660–5)

## Introduction

Moderate and florid ductal hyperplasia of the breast, defined as proliferation of epithelial cells that bridge or fill the ductal lumina, is a form of benign breast disease that has been associated with up to a doubling in risk of breast cancer. Hyperplasia accompanied by cytologic and/or architectural atypia increases risk by 5-fold (1). It is not yet clear if hyperplasia is a direct precursor of mammary carcinoma or is only a risk marker (2, 3).

Data from epidemiologic studies, cell culture systems, and animal models strongly implicate estrogens in the etiology of breast cancer (4). Androgens have also been linked to breast cancer risk in recent epidemiologic studies (5). The association between endogenous hormones and risk of mammary epithelial hyperplasia has not been well studied, but is of interest to determine when in the pathologic process towards breast cancer hormones are important. Most studies of endogenous hormones and benign breast conditions in postmenopausal women have found no significant differences in estrogen (6-10), androgen (9, 11), and sex hormone binding globulin (6-9) levels between cases with benign breast disease and controls. However, none of these studies focused specifically on epithelial hyperplasia, which has been estimated to constitute ~30% of benign specimens (12).

In this report, we assess the relationship between serum concentrations of estrogens, sex hormone binding globulin, and androgens and moderate or florid mammary hyperplasia with or without atypia in postmenopausal women. The control group consisted of women with nonproliferative breast histology not related to increased breast cancer risk.

## Materials and Methods

**Establishment of the Breast Serum Bank.** The development of the Mayo Serum Bank has been previously described (13). Between 1977 and 1987 all patients about to undergo breast biopsy or mastectomy in three hospitals in Grand Rapids, Michigan (henceforth referred to as hospitals A, B, and C) were invited to provide serum as part of a study to assess putative new breast cancer markers (14). In total, 5,358 women provided written informed consent and completed an in-person interview assessing breast cancer risk factors. We extracted pathologic diagnoses and information about extent of disease from medical records. Before surgery, volunteers donated 30 mL of nonfasting blood that was collected in sterile vacutainers, immediately chilled, and allowed to clot. The serum was separated within 2 hours and was then divided into 1 mL aliquots and stored at −70°C in sealed glass vials. The serum samples were then shipped in dry ice first to a central repository at the Mayo Foundation (Rochester, MN) and subsequently to the National Cancer Institute and stored at −70°C to −76°C (14).

**Subject Selection.** Selection criteria for study subjects included in this analysis are shown in Table 1. The table reflects the order in which the inclusion criteria were applied; the last row indicates the number of women who met all criteria.

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**Table 1. Selection criteria for study subjects**

| | | Benign breast disease subjects |
|---|---|---|
| Postmenopausal | | 1,375 (100%) |
| No diabetes or prior cancer | | 1,115 (81%)* |
| Not taking estrogen therapy or oral contraceptives | | 837 (61%)* |
| Blood draw on or several days before diagnosis, but >1 y after last menstrual period | | 769 (56%)* |
| 7 mL of serum available | | 707 (51%)* |
| Slides and pathology reports retrieved | | 607 (44%)* |
| | Control group | Hyperplasia case group |
| Meets criteria for case or control based on histology† | 159 | 187 |
| All analytes successfully measured | 152 | 179 |

*Percent of postmenopausal women.
†Excluded were 261 subjects with benign breast disease histologies that were not included in the control group or case group.

**Pathology Review.** We successfully retrieved slides and pathology reports for 607 subjects (86% of eligible subjects with sufficient serum available; Table 1). The primary study pathologist (M.E.S.) reviewed all histopathologic sections to confirm the benign diagnosis and further subclassify the histologic changes.

The potential case group defined by the review consisted of 187 subjects for whom slide review showed no breast cancer but indicated moderate or florid hyperplasia (proliferation more than four cell layers thick with bridging or distention of the lumen) with or without atypia; all hormones were successfully measured on 179 of these subjects. Of these 179, 42 had atypical hyperplasia. We excluded 113 subjects with mild hyperplasia (epithelial proliferation three to four layers thick which does not bridge or distend the lumen) from the case group because this condition is generally not associated with increased breast cancer risk (1).

The control group was selected from subjects with no breast cancer but with nonproliferative benign changes unassociated with increased breast cancer risk (3). A total of 159 women met these criteria on slide review; all hormones were successfully measured for 152 of these subjects. The controls had the following diagnoses: (*a*) nonproliferative changes not otherwise specified (50%); (*b*) atrophic lobules (31%); (*c*) apocrine metaplasia (14%); and (*d*) both apocrine metaplasia and atrophic lobules (5%). We excluded from the control group those with the following conditions: mild hyperplasia, microscopic papilloma, cysts $\geq$ 1 cm, sclerosing adenosis, adenosis, and fibroadenoma because these were either proliferative conditions similar to those in the case group or were histologic conditions that have been linked to a small increased risk of breast cancer in some studies (3).

Study subjects were diagnosed between 1977 and 1987 by community practice-based pathologists in Michigan. We used a pathology re-review to more consistently catalogue the observed changes. There were two breast pathologists in our investigative group. The primary study pathologist (M.E.S.) reviewed all the breast biopsies. A secondary, ''referee'' pathologist (C.M.)—without knowing the specific disagreement—reviewed all cases in which the primary study pathologist disagreed with the original pathologic diagnosis to the degree that the cases would be differently classified in the study (i.e., control versus hyperplasia case versus exclusion). Such differences occurred in 1% of the control group ($n$ = 2) and in 12% of the hyperplasia case group ($n$ = 22); the overall agreement was 93%. The referee pathologist concurred with the primary study pathologist in 16 (67%) nonconcordant cases. The final diagnosis was determined by the majority among the original diagnosis and the two slide reviews. Where there were three different assessments along a spectrum (e.g., nonproliferative disease, hyperplasia, and carcinoma *in situ*), we used the intermediate classification (in this example, hyperplasia). Three different assessments occurred for five of the study subjects with discrepancies.

Ninety-six percent of cases and controls were white. There were only minor differences in mean age at diagnosis, age at menopause, height, Quetelet Index, age at menarche, year of blood draw, and hour of blood draw between the 607 eligible subjects with benign changes and the otherwise eligible subjects excluded from the analyses only because we did not have their serum or slides ($n$ = 162).

**Serum Assays.** Serum levels of estrogens and sex hormone binding globulin were assayed in 2001 to 2002 at Esoterix, Inc. (Calabasas Hills, CA). Estradiol was measured by radioimmunoassay (RIA) after extraction and Sephadex LH20 column chromatography. The lower limit of quantitation, defined as the level at which the coefficient of variation is 20% or lower, was 18.0 pmol/L, although we were able to detect levels as low as 1.8 pmol/L. Percent bioavailable estradiol (the fraction of serum estradiol that is free or loosely bound to albumin) was determined after precipitation of the sex hormone binding globulin–bound steroid with ammonium sulfate. Total estradiol was multiplied by this percent to get the concentration of bioavailable estradiol. The lower limit of quantitation was 1.0 pmol/L. Estrone was measured by RIA after extraction and Sephadex LH20 column chromatography. The lower level of quantitation was 18.0 pmol/L. Estrone sulfate was measured by RIA after purification on Sephadex LH20 columns. The lower limit of quantitation was 286.0 pmol/L. Sex hormone binding globulin was assessed using an immunoradiometric assay. The lower limit of quantitation was 10.0 nmol/L.

Serum levels of testosterone, dehydroepiandrosterone, and androstenedione were assayed in 2001 at Quest Diagnostics (Van Nuys, CA). Testosterone and androstenedione were measured by RIA after extraction and chromatography. The lower limits of quantitation were 0.07 and 0.10 nmol/L, respectively. Dehydroepiandrosterone was measured by RIA after extraction; the lower limit of quantitation was 0.35 nmol/L. Androstenediol was measured by RIA after extraction and celite chromatography at the Reproductive Endocrinology Laboratory at the University of Southern California in 2001. The lower limit of quantitation was 0.14 nmol/L.

For each assay, samples from study subjects were randomly assigned to batches, with each batch containing approximately equal numbers of case and control samples. Two aliquots from each of two pooled quality control sera were randomly inserted in each batch. Laboratory personnel were unable to distinguish among case, control, and quality-control samples. Using a nested components of variance analysis, with logarithmically transformed quality control measurements (15), the estimated coefficients of variation (which take into account both within and between batch variation) of the assays were 30.7% for estradiol, 6.1% for bioavailable estradiol, 21.0% for estrone, 23.9% for estrone sulfate, 14.4% for sex hormone binding globulin, 8.6% for testosterone, 5.3% for dehydroepiandrosterone, 7.5% for androstenedione, and 9.8% for androstenediol.

**Statistical Methods.** We used unconditional logistic regression to estimate odds ratios (OR) and compute 95% confidence intervals (CI) for the risk of breast hyperplasia associated with serum hormone concentrations. For the primary analyses, hormone concentration categories were defined by quartiles of the frequency distribution in controls. To assess trends, we used the $P$ value from models with the $\log_e$-transformed values of hormone levels entered as a continuous variable. We adjusted all regression analyses for the following study design variables:

hospital, year of blood draw, and hour of the blood draw. We also adjusted all analyses for the two risk factors in these data that were associated with variations in hormone levels: age at diagnosis and nulliparity. Additionally, we adjusted selected analyses for Quetelet Index, which is an important determinant of estrogen levels. Adjustment for time since menopause did not appreciably alter the estimates. We included study hospital and nulliparity in the analyses as categorical variables and age at diagnosis, year of blood draw, hour of the blood draw, and Quetelet Index as continuous variables. All of these variables had complete data.

We calculated Pearson correlation coefficients ($r$) for the $\log_e$-transformed values of the serum hormone measurements. We used analysis of covariance to estimate geometric mean levels of the analytes adjusted to the mean age of the combined study subjects. For all analyses the preset level of statistical significance was 5% from a two-sided statistical test.

## Results

Twenty-six percent of controls and 32% of cases with hyperplasia were identified at hospital A. Forty-eight percent and 27%, respectively, were diagnosed at hospital B and 26% and 41%, respectively, were diagnosed at hospital C. The mean year of blood draw for the controls was 1981 and for the cases with hyperplasia was 1983.

Cases on average were almost 2 years older than controls ($P = 0.08$), had higher body mass indices ($P = 0.02$), and were less likely to be nulliparous ($P = 0.01$; Table 2). There were no significant differences between cases and controls for the other characteristics shown in Table 2.

Serum concentrations of the $\log_e$-transformed values of the estrogens were strongly and significantly correlated with each other among the controls ($r = 0.48$-$0.98$; Table 3). Testosterone was moderately correlated with the other three androgens ($r = 0.3$-$0.6$) and the other three androgens were very highly correlated with $r$ ranging from 0.7 to 0.9. Furthermore, estrogens and androgens were generally moderately and significantly correlated with each other ($r = 0.24$-$0.6$). Sex hormone binding globulin was not correlated with the androgens, but was negatively correlated with estradiol ($r = -0.17$), estrone sulfate ($r = -0.30$), and bioavailable estradiol ($r = -0.32$).

**Table 2. Selected characteristics of the study population**

| | Controls ($n$ = 152) | Cases with hyperplasia ($n$ = 179) | $P$ |
|---|---|---|---|
| | Mean (SD) | Mean (SD) | |
| Age at diagnosis (y) | 60.9 (10.1) | 62.7 (9.1) | 0.08* |
| Age at menopause (y) | 45.7 (7.5) | 46.1 (7.3) | 0.63* |
| Age at menarche (y)† | 12.9 (1.7) | 13.1 (1.6) | 0.41* |
| Height (cm) | 163.2 (6.1) | 163.3 (6.4) | 0.92* |
| Quetelet Index‡ | 25.0 (3.9) | 26.1 (4.7) | 0.02* |
| Nulliparous (%) | 19.1 | 9.5 | 0.01§ |
| Number of full-term pregnancies‖ | 3.2 (2.0) | 3.3 (1.6) | 0.91* |
| Age at first full-term pregnancy‖ | 23.7 (4.9) | 23.5 (4.3) | 0.55* |
| Family history of breast cancer (%)¶ | 28.3 | 26.3 | 0.92§ |

*$T$ test.
†Age at menarche was unknown for three controls and two cases with hyperplasia.
‡Weight (kg)/height (m)$^2$.
§$\chi^2$ test.
‖Among 123 parous controls and 162 parous cases.
¶Family history included mother, grandmother, sister, and aunt. Family history was unknown for five controls and six cases with hyperplasia.

Age-adjusted geometric mean serum concentrations of all estrogens were 27% to 50% higher in hyperplasia cases than controls, whereas those for sex hormone binding globulin were 15% lower (Table 4). There were no significant differences in mean androgen levels.

In logistic regression analyses, women in the two highest quartiles of estrone sulfate, estrone, estradiol, or bioavailable estradiol had statistically significant 2.5- to 5-fold increased risks of breast hyperplasia, relative to women in the lowest quartile of each estrogen; all tests for trend were highly significant (Table 5). Adjustment for sex hormone binding globulin and testosterone (potential risk factors which were correlated with the estrogens) did not substantially change these results (Table 5). Further adjustment for Quetelet Index did not appreciably alter any of the ORs (data not shown). Exclusion of subjects with extreme estrogen values also did not change the results. Associations with estradiol did not vary significantly when analyses were done separately for those at or below the median of Quetelet Index and those above the median.

There was a statistically significant inverse trend in risk of hyperplasia with sex hormone binding globulin levels ($P_{trend} = 0.05$). Additional adjustment for estradiol, which was correlated with sex hormone binding globulin ($r = -0.17$), somewhat attenuated the association ($P_{trend} = 0.10$). After adjustment for estrone sulfate, which was more strongly correlated with sex hormone binding globulin levels ($r = -0.30$), there was no reduction in risk in the fourth quartile of sex hormone binding globulin ($P_{trend} = 0.28$).

ORs for breast hyperplasia by quartiles of serum androgens are also shown in Table 5. Higher serum levels of testosterone were associated with elevated risk, but the trend in risk was not steady nor statistically significant ($P_{trend} = 0.34$). Adjustment for estradiol (for which testosterone is a precursor) and other estrogens (data not shown) attenuated these ORs. The slight elevations in risk associated with higher serum concentrations of other androgens did not generate statistically significant trends and were also noticeably attenuated after adjustment for estradiol or other estrogens. Even among women in the lowest tertile of serum estradiol, neither testosterone nor androstenediol was clearly associated with risk of hyperplasia, but these results were based on small numbers.

To assess the independent contribution of the different estrogens to risk, we focused on estradiol and estrone sulfate because they were strongly associated with risk and least correlated with each other. The ORs associated with the three upper quartiles of estradiol after adjustment for estrone sulfate in addition to the other variables were 1.5 (95% CI, 0.7-3.2), 1.6 (95% CI, 0.7-3.6), and 2.3 (95% CI, 1.0-5.6; $P_{trend} = 0.17$). The ORs for estrone sulfate after adjustment for estradiol were 1.8 (95% CI, 0.8-4.2), 3.3 (95% CI, 1.4-7.7), and 2.9 (95% CI, 1.1-7.3; $P_{trend} = 0.04$).

To further investigate the combined effects of the estrogens, we compared women in the highest tertiles of estradiol, estrone, and estrone sulfate with those in the lowest tertile of all three hormones. There were 54 cases and 30 controls in the highest tertile, 114 cases and 97 controls in the middle tertile, and 11 cases and 25 controls in the lowest tertile. The OR associated with the highest tertile compared with the lowest tertile was 5.8 (95% CI, 2.2-15.2) and for all other categories compared with the lowest tertile was 3.3 (95% CI, 1.4-7.7) after adjustment for the study design variables, age at diagnosis, and nulliparity. The ORs for the highest compared with the lowest tertile for the hormones individually were 3.5 (95% CI, 1.8-6.7) for estradiol, 3.4 (95% CI, 1.8-6.3) for estrone, and 4.4 (95% CI, 2.2-8.6) for estrone sulfate.

**Table 3. Pearson correlation coefficients and *P* values among log-transformed values of analytes among the controls**

| | Bioavailable estradiol | Estrone | Estrone sulfate | Sex hormone binding globulin | Testosterone | Dehydroepian-drosterone | Androstenedione | Androstenediol |
|---|---|---|---|---|---|---|---|---|
| Estradiol | 0.98 | 0.53 | 0.48 | −0.17 | 0.31 | 0.34 | 0.43 | 0.37 |
| | <0.0001 | <0.0001 | <0.0001 | 0.04 | <0.0001 | <0.0001 | <0.0001 | <0.0001 |
| Bioavailable estradiol | | 0.54 | 0.53 | −0.32 | 0.31 | 0.36 | 0.44 | 0.37 |
| | | <0.0001 | <0.0001 | <0.0001 | 0.0001 | 0.0007 | 0.0001 | 0.0001 |
| Estrone | | | 0.73 | −0.09 | 0.42 | 0.52 | 0.60 | 0.52 |
| | | | <0.0001 | 0.28 | <0.0001 | <0.0001 | <0.0001 | <0.0001 |
| Estrone sulfate | | | | −0.30 | 0.24 | 0.43 | 0.42 | 0.40 |
| | | | | 0.0002 | 0.0029 | <0.0001 | <0.0001 | <0.0001 |
| Sex hormone binding globulin | | | | | 0.03 | −0.05 | −0.02 | 0.05 |
| | | | | | 0.73 | 0.56 | 0.80 | 0.53 |
| Testosterone | | | | | | 0.33 | 0.56 | 0.36 |
| | | | | | | <0.0001 | <0.0001 | <0.0001 |
| Dehydroepiandrosterone | | | | | | | 0.81 | 0.90 |
| | | | | | | | <0.0001 | <0.0001 |
| Androstenedione | | | | | | | | 0.70 |
| | | | | | | | | <0.0001 |

## Discussion

We compared serum concentrations of estrogens, sex hormone binding globulin, and androgens among postmenopausal women with moderate/florid hyperplasia with or without atypia to hormone concentrations among postmenopausal women with nonproliferative histologic changes in the breast that are not associated with increased breast cancer risk. We found that higher serum levels of estrogens, including estradiol, bioavailable (non sex hormone binding globulin bound) estradiol, estrone, and estrone sulfate, were strongly and significantly associated with increased risk of hyperplasia. Moreover, estradiol and estrone sulfate were each associated with some elevation in risk after adjustment for the other. Although estrone sulfate is biologically inactive, it serves as a reservoir for the biosynthesis of the more potent estradiol in the mammary gland (16). Women with high levels of all three hormones (estradiol, estrone, and estrone sulfate) were at nearly six times the risk of those with low levels of all three hormones. The associations we report are somewhat stronger than those generally reported for breast cancer (5).

In this study, serum sex hormone binding globulin levels were not associated with a reduction in risk of hyperplasia after adjustment for estrogen levels. Increasing levels of sex hormone binding globulin have been associated with reduced postmenopausal breast cancer risk, with the magnitude of the associations slightly reduced after adjustment for estradiol (5). We also found no significant associations between serum levels of androgens and risk of hyperplasia.

Prior studies have not shown strong or significant differences in levels of estrogens (6-10), androgens (9, 11), or sex hormone binding globulin (6-9) in postmenopausal patients with and without benign breast disease. Most of the published studies have been based on small sample sizes (6, 9, 10), did not distinguish between proliferative and nonproliferative changes (7, 8), and did not include a rigorous pathology review (6-10). All of these studies were done at a time when endogenous hormone assays were notably less reliable and accurate than they are now.

Normal breast epithelium contains a low percentage of cycling cells, the majority of which do not express estrogen receptors (17). However, hyperplastic lesions show an increased number of dividing cells, many of which are receptor positive (18, 19). Accordingly, up-regulation of estrogen receptor in combination with increased exposure to circulating estrogens represents a plausible model for the development of hyperplasia. The lower risk of breast hyperplasia among women who use tamoxifen (20) is also consistent with a role for estrogens in the development of this condition. Some studies have found that a subset of hyperplastic lesions are clonal and share molecular alterations with coexisting carcinomas in the same breast (21). However, the biology of hyperplasia is only minimally understood and it is not certain whether most lesions represent direct precursors of cancer at the tissue level or are simply a marker of a high-risk predispositional state (2).

Although testosterone and other androgens have been linked to increased breast cancer risk (5), testosterone has not shown a stimulatory effect on mammary epithelial cells in cell culture experiments (22) and has inhibited mammary epithelial proliferation and suppressed estrogen receptor expression in animal models (23). Thus, our results add to the aggregate of data that androgens have little, if any, role in benign breast changes. Perhaps the lack of association

**Table 4. Age-adjusted geometric mean serum concentrations for estrogens, sex hormone binding globulin, and androgens in controls and cases**

| | Controls | Cases | *P** |
|---|---|---|---|
| | Mean (95% CI) | Mean (95% CI) | |
| Estradiol (pmol/L) | 17.2 (14.3-20.9) | 25.3 (21.3-29.7) | 0.004 |
| Bioavailable estradiol (pmol/L) | 7.7 (6.2 9.2) | 11.7 (9.9-14.3) | 0.0009 |
| Estrone (pmol/L) | 88.8 (81.4-96.2) | 114.6 (103.6-122.0) | 0.0001 |
| Estrone sulfate (pmol/L) | 1,150.2 (1,041.5-1,270.4) | 1,462.1 (1,336.2-1,602.3) | 0.0006 |
| Sex hormone binding globulin (nmol/L) | 103.7 (94.7-113.5) | 88.6 (81.5-96.3) | 0.04 |
| Testosterone (nmol/L) | 0.7 (0.6-0.8) | 0.7 (0.6-0.8) | 0.39 |
| Dehydroepiandrosterone (nmol/L) | 9.2 (8.3-10.2) | 9.7 (8.8-10.7) | 0.71 |
| Androstenedione (nmol/L) | 2.8 (2.6-3.0) | 2.9 (2.7-3.1) | 0.67 |
| Androstenediol (nmol/L) | 1.4 (1.3-1.6) | 1.5 (1.4-1.6) | 0.90 |

*Analysis of covariance.

**Table 5. ORs (95% CIs) for breast hyperplasia associated with quartiles of serum concentrations of estrogens, sex hormone binding globulin, and androgens in postmenopausal women**

| | $Q_1$* | $Q_2$ | $Q_3$ | $Q_4$ | $P_{trend}$† |
|---|---|---|---|---|---|
| Estradiol (pmol/L) | ≤11.01 | 11.02-20.19 | 20.20-29.37 | >29.37 | |
| (Cases/controls) | (23/43) | (52/39) | (37/33) | (67/37) | |
| OR‡ | 1.0 | 2.2 (1.1-4.6) | 2.5 (1.1-5.3) | 4.1 (2.0-8.5) | 0.007 |
| OR‡,§ | 1.0 | 2.0 (0.9-4.3) | 2.1 (1.0-4.8) | 3.9 (1.7-8.9) | 0.02 |
| Bioavailable estradiol (pmol/L) | ≤4.41 | 4.42-8.08 | 8.09-15.05 | >15.05 | |
| (Cases/controls) | (19/38) | (35/38) | (58/38) | (67/38) | |
| OR‡ | 1.0 | 2.0 (0.9-4.4) | 3.5 (1.6-7.6) | 4.5 (2.1-10.0) | 0.003 |
| OR‡,‖ | 1.0 | 1.9 (0.8-4.3) | 3.3 (1.5-7.4) | 4.3 (1.9-9.5) | 0.008 |
| Estrone (pmol/L) | ≤62.87 | 62.88-90.61 | 90.62-133.14 | >133.14 | |
| (Cases/controls) | (19/40) | (35/39) | (74/35) | (51/38) | |
| OR‡ | 1.0 | 2.0 (0.9-4.4) | 4.2 (2.0-8.9) | 3.1 (1.4-6.6) | 0.0004 |
| OR‡,§ | 1.0 | 1.8 (0.8-4.1) | 3.5 (1.5-7.7) | 2.7 (1.2-6.4) | 0.0006 |
| Estrone sulfate (pmol/L) | ≤776.82 | 776.83-1,065.80 | 1,065.81-1,700.27 | >1,700.27 | |
| (Cases/controls) | (18/38) | (35/39) | (67/37) | (59/38) | |
| OR‡ | 1.0 | 2.1 (1.0-4.8) | 4.3 (2.0-9.4) | 4.6 (2.0-10.4) | 0.0009 |
| OR‡,§ | 1.0 | 2.2 (0.9-5.2) | 4.8 (2.1-11.2) | 5.0 (2.0-12.3) | 0.0048 |
| Sex hormone binding globulin (nmol/L) | ≤76.73 | 76.74-102.30 | 102.31-135.85 | >135.85 | |
| (Cases/controls) | (74/38) | (33/38) | (30/38) | (42/38) | |
| OR‡ | 1.0 | 0.4 (0.2-0.8) | 0.4 (0.2-0.8) | 0.7 (0.4-1.4) | 0.05 |
| OR‡,¶ | 1.0 | 0.4 (0.2-0.8) | 0.4 (0.2-0.9) | 0.8 (0.4-1.7) | 0.10 |
| OR‡,** | 1.0 | 0.4 (0.2-0.8) | 0.4 (0.2-0.9) | 1.2 (0.6-2.5) | 0.28 |
| Testosterone (nmol/L) | ≤0.45 | 0.46-0.66 | 0.67-1.00 | >1.00 | |
| (Cases/controls) | (36/39) | (41/43) | (59/34) | (43/36) | |
| OR‡ | 1.0 | 1.3 (0.6-2.6) | 2.3 (1.1-4.5) | 1.4 (0.7-2.8) | 0.34 |
| OR‡,¶ | 1.0 | 1.1 (0.5-2.3) | 1.7 (0.8-3.5) | 0.9 (0.4-1.9) | 0.89 |
| Dehydroepiandrosterone (nmol/L) | ≤6.30 | 6.31-10.50 | 10.51-15.50 | >15.50 | |
| (Cases/controls) | (45/38) | (54/38) | (36/38) | (44/38) | |
| OR‡ | 1.0 | 1.5 (0.7-2.9) | 0.9 (0.4-1.8) | 1.2 (0.6-2.5) | 0.65 |
| OR‡,¶ | 1.0 | 1.1 (0.6-2.3) | 0.7 (0.3-1.4) | 0.8 (0.3-1.7) | 0.65 |
| Androstenedione (nmol/L) | ≤2.10 | 2.11-2.86 | 2.86-4.26 | >4.26 | |
| (Cases/controls) | (39/38) | (49/39) | (47/37) | (44/38) | |
| OR‡ | 1.0 | 1.0 (0.5-2.0) | 1.4 (0.7-2.8) | 1.2 (0.6-2.5) | 0.58 |
| OR‡,¶ | 1.0 | 0.8 (0.4-1.7) | 1.0 (0.5-2.2) | 0.8 (0.4-1.8) | 0.64 |
| Androstenediol (nmol/L) | ≤1.03 | 1.04-1.62 | 1.63-2.23 | >2.23 | |
| (Cases/controls) | (46/38) | (53/38) | (33/39) | (47/37) | |
| OR‡ | 1.0 | 1.7 (0.9-3.4) | 0.9 (0.4-1.9) | 1.9 (0.9-4.0) | 0.22 |
| OR‡,¶ | 1.0 | 1.4 (0.7-2.8) | 0.6 (0.3-1.3) | 1.1 (0.5-2.6) | 0.81 |

*Reference category.
†*P* value for trend from model with the logarithm of hormone level entered as a continuous variable.
‡Adjusted for age at diagnosis, hospital, year of blood draw, hour of blood draw, and nulliparity.
§Adjusted for quartiles of sex hormone binding globulin and testosterone.
‖Adjusted for quartiles of testosterone.
¶Adjusted for quartiles of estradiol.
**Adjusted for quartiles of estrone sulfate.

reflects a dual role of androgens as inhibitors of cellular proliferation and as precursors of estrogens, which are associated with an increased risk of hyperplasia in our study.

We would like to highlight several aspects of the study method. The control group in the present study consisted of patients who, like the cases, had undergone breast biopsy; in fact, case and control groups were distinguished on the basis of the histologic findings. This design ensured that the control group did not have identifiable mammary hyperplasia in the biopsy—the attribute that defined the cases—nor did they have other breast conditions that have been associated with increased breast cancer risk, such as fibroadenoma, sclerosing adenosis, or macrocysts.

Because this was a case-control study, it is possible that the benign breast changes themselves affected circulating concentrations of the measured analytes. However, this seems unlikely because benign breast hyperplasia is localized to breast epithelium and is unlikely to generate systemic effects. We adjusted for year of blood draw to account for any possible degradation in the analytes over time and adjusted for hour of blood draw to adjust for potential diurnal variation. Another limitation is that we did not have information on prior exogenous hormone use. If participants had recently quit, it is possible that serum levels could reflect recent exogenous hormone use, although there is no reason to suspect that this might differ for cases and controls. Moreover, selective exclusion of participants with extreme estrogen values did not change the results.

The relatively large coefficients of variation for estradiol, estrone, and estrone sulfate, which reflect the limited precision of the assays, are of some concern. However, the differences between cases and controls for these analytes were sufficiently large compared with the laboratory variability that we still were able to identify statistically significant associations (15). The fact that we found higher ORs when we identified women who were concurrently high or low on all three estrogens (estradiol, estrone, and estrone sulfate) may indicate that multiple measurements with distinct assay kits more accurately identify women at highest and lowest risk. It is noteworthy that we found no associations for the androgens, which had the lowest coefficients of variation.

Finally, we do not know whether the volunteers who gave blood for the breast cancer marker study in the 1970s and 1980s differed from those who did not because we did not collect information from this latter group. We had to exclude some women who met the eligibility criteria for the study but for whom we lacked serum or breast biopsy slides. However, there were only minor differences in measured variables between those included and those excluded.

In summary, we found that higher serum estrogen levels—but not androgen or sex hormone binding globulin levels—were strongly associated with moderate or florid hyperplasia with or without atypia, breast cancer risk factors, and possible precursors, associated with at least a 2-fold increase in breast cancer risk. Our findings for estrogens are consistent with results for breast cancer. Our results suggest that androgens overall are not influential at this stage of breast pathology. Associations between sex hormone binding globulin and androgens and breast cancer in other studies suggest the possibility that they act at a later stage or on another pathway in the development of breast cancer.

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